Vol. 44 No. 12

BULLETIN

OF THE

TORREY BOTANICAL CLUB

DECEMBER, 1917

# The development of the embryo-sac and of the embryo in Phaseolus vulgaris

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(WITH PLATES 25 and 26)

## INTRODUCTION

So far as I have been able to find, the only previously published description of the embryo-sac or embryo of *Phaseolus* is by Guignard (1881), who studied forty species of Leguminosae, among them *Phaseolus multiflorus*. In this species he found that an axial row of but three macrospores is formed. The innermost cell of this row by three successive nuclear divisions forms the embryo-sac, which is typical in every respect; the antipodals are ephemeral. The first division of the egg is transverse, and occurs at the same time that the primary endosperm nucleus divides. A pro-embryo of three cells is formed, the terminal one of which develops into the embryo; the other cells form the suspensor. Divisions follow until an embryo is formed at the apex of a filamentous suspensor, which is two cells in thickness and whose basal cells are conspicuously swollen.

In the present study buds, pistils, and developing fruits were obtained from the following varieties of *Phaseolus vulgaris:* "David Kidney," "Longfellow," "Pole," and "Kidney Wax," plants of which were grown in the greenhouse during the fall and winter of 1914–15 and 1915–16, and "Wardwell's Wax," grown in the

[The BULLETIN for November (44: 501–534. *pl. 24*) was issued November 20, 1917]

garden during the summer of 1915. Material was collected from the time the buds began to appear until the fruits were fully grown.

The very young buds were placed in the fixing fluids intact; the floral envelopes were removed from the larger buds and flowers to insure penetration of the fixing fluids. For the same reason the pods were cut into pieces, and in the case of the older pods the young seeds were picked out and placed separately in the fixing solutions. Flemming's solutions, medium and strong; 1 per cent. chrom-acetic acid, Juel's fluid and Carnoy's fluid were used as fixatives. The best preparations showing the development of the macrospores and the embryo-sac were obtained from material fixed in Flemming's medium solution and in chrom-acetic acid. Juel's and Carnoy's fluids gave best results in the fixation of embryos.

Longitudinal sections of these various structures were cut from five to twelve microns in thickness. Flemming's triple stain, Heidenhain's iron-alum haematoxylin, and a combination of the latter with Lichtgrün were used.

I have found no differences between any of the varieties used, so far as the history of the embryo-sac and the embryo are concerned; the description which follows applies equally, therefore, to all the varieties named.

## THE MACROSPORES AND THE EMBRYO-SAC

In the varieties of *Phaseolus vulgaris* studied, from two to seven ovules are borne in an apparently single row upon the adherent edges of the carpel. When first formed the ovules are orthotropous, but as growth proceeds they become recurved and are campylotropous when mature.

The two integuments, when fully grown, surround the ovule on all sides, but are slightly shorter on the side toward the placenta. They grow rapidly and by the time that the macrospore mother cell is fully grown the outer integument has reached almost to the apex of the ovule, the inner one being at this time about two thirds the length of the outer integument.

A hypodermal cell in the axial row of the nucellus becomes larger than the surrounding cells and stands out conspicuously

among them (PLATE 25, FIG. 1). This cell is typically five-sided in section; it contains a very large nucleus and stains more deeply than the adjacent cells. At a later stage the young macrospore mother cell is separated from the epidermal layer by another layer of cells (FIG. 4). Still later, when the macrospore mother cell is fully grown, there are often two layers of cells between it and the epidermis (FIG. 5); this is not always the case, however, for not infrequently the elongated macrospore mother cell is in the third instead of the fourth layer of cells. No division figures were seen either in the first differentiated hypodermal cell or in the cells of the epidermal layer. For this reason, I have been unable to determine whether the hypodermal cell that is early distinguished by its size itself functions as the macrospore mother cell, or whether, on the other hand, this hypodermal cell divides, one of its daughter cells becoming the macrospore mother cell. On the whole, the arrangement of the subepidermal layers at the later stages (FIG. 5) supports rather more strongly the former hypothesis. Preparations were obtained in which there were what seemed to be two young macrospore mother cells in an axial row (FIG. 3); another preparation showed two young mother cells lying side by side, and in one case two fully grown mother cells lay side by side. In no case, however, was the further development of more than one macrospore mother cell observed.

The fully grown macrospore mother cell is about three times as long as wide (FIG. 6); its nucleus is near the micropylar end of the cell; the chalazal end is usually pointed (FIG. 6), but in some cases quite rounded (FIG. 7). The nucleus remains in the micropylar end of the cell during the prophases of the ensuing division. FIG. 7 shows the nucleus in synapsis. One preparation was obtained showing the heterotypic division; the spindle lies approximately in the center of the cell (FIG. 8). One of the two daughter cells formed by this division fails to undergo a second division, since, so far as my preparations show, a row of but three macrospores is formed (FIG. 9). Guignard (1881) reported the formation of but three macrospores in *Phaseolus multiflorus* but did not determine which of the two daughter cells, formed from the division of the mother cell, fails to divide; he also found a case in which a longitudinal division took place in one of the functionless macrospores;

the innermost macrospore at the chalazal end develops into the embryo-sac, and I find the same thing true in *Phaseolus vulgaris*. In other Leguminosae, according to Guignard, two, three, or four macrospores may be formed and either the innermost spore or the one next it may develop into the embryo-sac. Saxton (1907) finds in *Cassia tomentosa* a deeply buried macrospore mother cell whose division forms a row of four macrospores, of which the one next to the innermost produces the embryo-sac. However, according to Martin (1914), in *Medicago sativa*, *Vicia americana*, and several species of *Trifolium* examined by him, an axial row of four macrospores is formed.

The functional macrospore becomes several times as long as wide before its nucleus divides (PLATE 26, FIG. 11). The two outer macrospores degenerate rapidly, and by the time that the developing macrospore reaches the binucleate stage they have usually disappeared entirely.

After the first nuclear division in the functional macrospore, the two daughter nuclei pass to the respective ends of the sac (FIG. 12), and a large vacuole appears between them. After the second nuclear division each end of the developing macrospore contains a pair of nuclei (FIG. 13). The nuclei at the micropylar end usually remain close together, those at the chalazal end being further apart. After the third division, a group of four nuclei is seen at each end of the embryo-sac.

The polar nuclei begin their migration very soon after the completion of the last nuclear division. Cell division ensues, resulting in the formation of a typical seven-celled embryo-sac (FIG. 14). The egg apparatus presents the usual appearance; the polar nuclei lie a short distance away from the egg apparatus in the median line; at the stage shown in FIG. 14 they have not yet begun to fuse. The antipodal cells are typically triangular in section, and their nuclei are smaller than the other nuclei of the sac. A large vacuole is characteristic of the embryo-sac at this stage, lying between the polar nuclei and the antipodal cells. This development seems to agree, except in minor details, with that of other Leguminosae that have been investigated. Saxton (1907) found an absorptive tissue derived from the antipodal cells in *Cassia tomentosa;* Hofmeister (1858) failed to find antipodal cells in the members

of the family studied by him; Hegelmaier (1880), also, did not see the antipodal cells and seems to have confused endosperm cells with the egg apparatus; according to Guignard (1881) the antipodal cells of *Phaseolus multiflorus* are ephemeral; and Strasburger (1880) found that the same is true of the antipodal cells of four species of *Lupinus*.

The egg grows after its formation (PLATE 25, FIG. 10) and becomes long and broad at the base so that it projects into the embryo-sac beyond the synergids (PLATE 26, FIG. 15). The egg nucleus becomes larger and a large vacuole appears in the cytoplasm toward the micropylar end of the egg. The synergids develop a distinct filiform apparatus (FIG. 15) whose striations arise from the neighborhood of a vacuole at the broader end of each synergid. Martin (1914) described a filiform apparatus in *Trifolium pratense*, but with more conspicuous striations than those which I have observed in *Phaseolus vulgaris*. The synergids change from a pear-shaped to a more narrow tapering form. After fertilization the synergids disintegrate and entirely disappear.

The polar nuclei come to lie close together just below the egg (FIG. 14) and remain in this position for some time. Before fertilization, however, they come into close contact with each other and begin to fuse (FIG. 16), but it is quite possible that their fusion may not be completed before the male nuclei enter the sac and one of the latter fuses with the polar nuclei.

In one preparation a pollen tube was seen entering the embryo-sac. It grows through the micropyle and pushes into the embryo-sac between the cells which form a sheath around the micropylar end of the sac.

The nucellus is gradually absorbed during the development of the embryo-sac, and when the latter is mature the nucellar tissue entirely disappears from the micropylar end and from the sides, leaving these parts of the sac in immediate contact with the inner integument; at the chalazal end of the sac, however, the nucellar tissue persists, its cells grow larger and become arranged in quite definite rows which seem to diverge from the point where the integuments arise from the nucellus. This tissue persists until, late in the history of the embryo-sac, it is finally absorbed. According to Hegelmaier (1880), the nucellus in the ovule of *Lupinus*

is entirely absorbed after fertilization; Ward (1881) notes the deliquescence of the cells surrounding the embryo-sac of *Lupinus venustus;* and Martin (1914) made similar observations on *Medicago sativa, Vicia americana*, and several species of *Lupinus*.

## THE EMBRYO

The first division of the fertilized egg is transverse (FIG. 18); the basal one of the two cells so formed typically encloses a large vacuole. The next division occurs in this basal cell and is transverse (FIG. 19), so that a filamentous pro-embryo of three cells is invariably formed (FIG. 20). The third division is a longitudinal one (FIG. 21), in the terminal cell; it is quickly followed by longitudinal divisions in the other two cells of the pro-embryo, which are to give rise to the suspensor (FIG. 22). Divisions may now occur in the longitudinal plane perpendicular to that just described, or the divisions in the second longitudinal plane may be preceded by several transverse divisions. The embryo then consists of four rows of seven or eight cells each (FIG. 23); and the basal cells have begun to show evidences of swelling.

Division now ceases except in the cells at the distal end of the embryo. Anticlinal walls are put in (FIG. 24, *a*) in the cells of the terminal tier. Periclinal walls are next formed, cutting off an outer layer of cells, the dermatogen (FIG. 26). In *Medicago sativa, Vicia americana*, and several species of *Trifolium*, Martin (1914) observed that the dermatogen is cut off later than the octant stage; Guignard (1881) also found this true in his work on *Phaseolus multiflorus*. When the embryo is about six or seven cells in length (FIG. 24), the four basal cells of the suspensor become swollen and turgid and much elongated; later the next tier of four cells above them also undergo like changes (FIG. 25). The swollen cells at the base of the suspensor continue to grow in length and retain their inflated appearance until late in the history of the embryo; but when the embryo has grown so as almost to fill the cavity of the sac, its growth seems to cause a compression of the basal cells and they become flattened in the micropylar end of the embryo-sac (FIG. 27). Swollen suspensor cells occur in other members of the Leguminosae; Hegelmaier (1880) reported such cells in the embryo of *Lupinus*, but in this case the cells were

multinucleate; Strasburger (1880) observed swollen suspensor cells in *Lupinus*, which in some instances show a tendency to separate from one another. Guignard (1881) also reported the occurrence of inflated cells which are multinucleate in *Orobus aureus*, *O. angustifolius*, and *Pisum sativum*, and suspensor cells which become separated from one another in *Lupinus polyphyllus*. Martin (1914) observed instances in his studies in which the suspensor cells retained their normal appearance and also cases in which the modifications which were noted by other investigators occurred.

At the time of the differentiation of the dermatogen, the embryo proper is almost ovoid in shape (FIG. 26); it retains this form as it increases in size until the appearance of the cotyledons. Cotyledon development begins later than the stage shown in Figure 26; but none of my preparations show satisfactorily the first stages in this development because the plane of the union of the cotyledons is parallel to the flat side of the ovule, and the embryo lies curved in the micropylar end of the embryo-sac. The embryo continues to grow at the expense of the endosperm and of the cushion of nucellar tissue at the chalazal end of the embryo-sac. The nucellus is absorbed, and by the time the embryo is mature the endosperm also has entirely disappeared.

## THE ENDOSPERM

The division of the primary endosperm nucleus as a rule precedes that of the fertilized egg (FIG. 17), although one preparation showed the egg nucleus and the primary endosperm nucleus dividing simultaneously. In *Phaseolus multiflorus*, Guignard (1881) found that the division of the egg nucleus and that of the primary endosperm nucleus occur at the same time; Strasburger (1880) observed the simultaneous division of these two nuclei in *Lupinus*; but Martin (1914) found that the first division of the fertilized egg is usually preceded by the first division of the primary endosperm nucleus.

The endosperm nuclei resulting from the first two divisions arrange themselves in the periphery of the embryo-sac. Usually two of them are to be seen near the young embryo, one on either side of it. The later nuclear divisions are not always simulta-

neous, for in several instances both resting nuclei and nuclei in the various stages of division are to be seen distributed from one end of the sac to the other (FIG. 28); but the order of their arrangement was not identical in the different sacs observed.

The endosperm remains in the form of a peripheral layer in the embryo-sac until its final absorption by the growing embryo; it is typically thicker around the embryo than in other regions of the sac. After the differentiation of the dermatogen, cell division occurs in that part of the endosperm which immediately surrounds the embryo itself. Each nucleus in this part of the endosperm is surrounded by a very definite cell wall; the cells so formed, however, do not form a compact mass but lie isolated in the undivided cytoplasm (FIG. 29). These cells do not persist but are soon absorbed, and all traces of the endosperm have entirely disappeared by the time of the maturity of the seed.

## SUMMARY

1. A large hypodermal cell is early differentiated in the ovule; this either functions as the macrospore mother cell or possibly divides once, one of its daughter cells being the macrospore mother cell.

2. The fully grown macrospore mother cell lies in either the third or fourth layer from the micropylar end of the nucellus.

3. An axial row of three macrospores is formed, the innermost of which develops into the embryo-sac.

4. The nucellus is entirely destroyed at the micropylar end and along the sides by the development of the embryo-sac; the nucellar tissue at the base of the sac takes on a peculiar structure and persists for some time, but finally is itself gradually absorbed by the embryo-sac.

5. The polar nuclei begin to approach each other soon after the eight-nucleate stage of the embryo-sac is reached and remain close together for some time just below the egg; then their fusion takes place.

6. The three antipodal cells disappear at about the time of fertilization.

7. The synergids form a conspicuous filiform apparatus

8. The pro-embryo consists of a filament of three cells; the

two basal cells form the suspensor and the terminal cell develops into the embryo proper.

9. The dermatogen is cut off when the embryo proper consists of about sixteen cells. When the suspensor consists of four rows of about seven or eight cells each, the two tiers of cells at its base become swollen and conspicuously elongated.

10. The primary endosperm nucleus usually divides before the first division of the egg; two of the daughter nuclei resulting from the first two divisions place themselves on either side of the young embryo; and in succeeding divisions the endosperm nuclei place themselves in the peripheral region of the embryo-sac.

11. The divisions of the endosperm nuclei may be simultaneous, or nuclei in all stages of division may be found at the same time, from resting nuclei at one end of the endosperm to late telophases at the opposite end.

12. Endosperm cells are formed in the region immediately about the embryo, but are later absorbed.

I wish to express my sincere appreciation to Dr. C. E. Allen, who suggested this work and under whose supervision it was done.

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BIBLIOGRAPHY

**Guignard L.** (1881). Recherches d'embryogénie végétale comparée. 1er Mémoire: Légumineuses. Ann. Sci. Nat. Bot. VI. **12**: 5–166. *pl. 1–8.*

**Hegelmaier, F.** (1880). Zur Embryogenie und Endospermentwickelung von *Lupinus*. Bot. Zeit. **38**: 65–73, 81–91, 97–103, 121–138, 143–149. *pl. 1, 2.*

**Hofmeister, W.** (1858). Neuere Beobachtungen über Embryobildung der Phanerogamen. Jahrb. Wiss. Bot. **1**: 101–103. *f. 1–41.*

**Martin, J. N.** (1914). Comparative morphology of some Leguminosae. Bot. Gaz. **58**: 154–167. *pl. 8–11.*

**Saxton, W. T.** (1907). On the development of the ovule and embryo-sac in *Cassia tomentosa*. Trans. So. Afr. Phil. Soc. **18**: 1–5. *pl. 1, 2.*

**Strasburger, E.** (1880). Einige Bemerkungen über vielkernige Zellen und über die Embryogenie von *Lupinus*. Bot. Zeit. **38**: 845–854, 857–868. *f. 1–64.*

**Ward, H. M.** (1881). A contribution to our knowledge of the embryo-sac in Angiosperms. Jour. Linn. Soc. Bot. **17**: 519–532.

## Explanation of plates 25 and 26

All drawings were made with an Abbé camera lucida at table level. Leitz oculars and objectives were used: FIGS. 1, 2, 6–10, 14–20, 22, with ocular 4, oil immersion objective 1/16, tube length 222 mm. (× 2,475); FIGS. 4, 5, 11–13, 21, 23, 25, with ocular 4, oil immersion objective 1/16, tube length 170 mm. (× 1,740); FIGS. 24, 26, 28, with ocular 3, oil immersion objective 1/16, tube length 170 mm. (× 1,530); FIG. 3, with ocular 1, oil immersion objective 1/16, tube length 140 mm. (× 966); FIG. 27, with ocular 3, objective 3, tube length 170 mm. (× 170); FIG. 29, with ocular 3, oil immersion objective 1/16, tube length 140 mm. (× 570). The drawings on PLATE 25 have been reduced one half in reproduction; those on PLATE 26, two thirds.

PLATE 25

FIG. 1. Young nucellus showing enlarged hypodermal cell.

FIG. 2. Young macrospore mother cell.

FIG. 3. Nucellus showing two macrospore mother cells in an axial row.

FIG. 4. Nucellus showing the position of the young macrospore mother cell represented on a larger scale in FIG. 2.

FIG. 5. Nucellus showing two layers of cells between the epidermal layer and the macrospore mother cell.

FIG. 6. Fully grown macrospore mother cell.

FIG. 7. Macrospore mother cell with nucleus in synapsis.

FIG. 8. Macrospore mother cell with nucleus in division.

FIG. 9. Row of three macrospores; the two upper ones are degenerating, the third has enlarged and will develop into the embryo-sac.

FIG. 10. Mature egg.

PLATE 26

FIG. 11. Functional macrospore with the two degenerating macrospores lying above it in the nucellus.

FIG. 12. Binucleate embryo-sac.

FIG. 13. A four-nucleate embryo-sac.

FIG. 14. A mature embryo-sac.

FIG. 15. Egg and synergids showing filiform apparatus.

FIG. 16. Polar nuclei fusing.

FIG. 17. An egg and two endosperm nuclei.

FIG. 18. A two-celled pro-embryo.

FIG. 19. The second division in the pro-embryo.

FIG. 20. A three-celled pro-embryo.

FIG. 21. The terminal cell of the pro-embryo has divided longitudinally.

FIG. 22. An embryo consisting of six cells.

FIG. 23. An embryo after further transverse divisions have occurred; the basal cells are enlarging.

FIG. 24. An embryo showing anticlinal walls in the terminal cells.

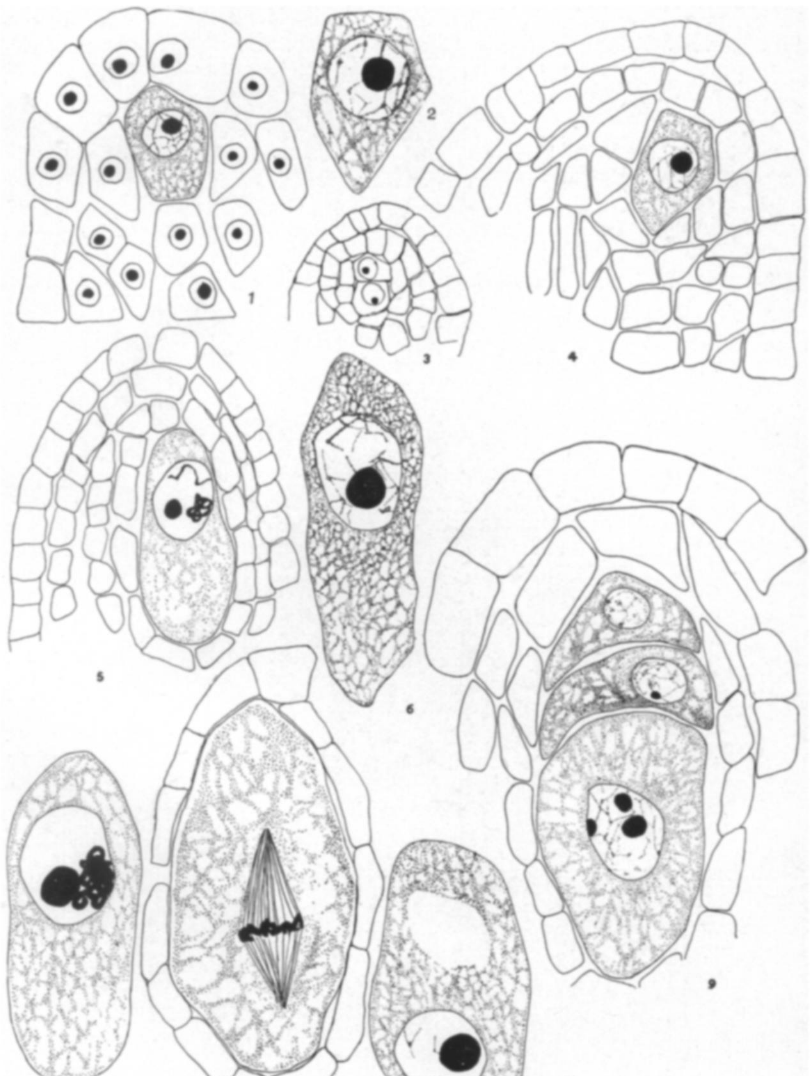
FIG. 25. Periclinal walls in the embryo proper.

FIG. 26. An older embryo showing suspensor with no distinct demarcation between it and the embryo proper.

FIG. 27. Embryo showing cotyledon, hypocotyl, and epicotyl. The basal cells are compressed against the integument.

FIG. 28. Endosperm nuclei dividing.

FIG. 29. Endosperm cells formed in the region of the embryo.

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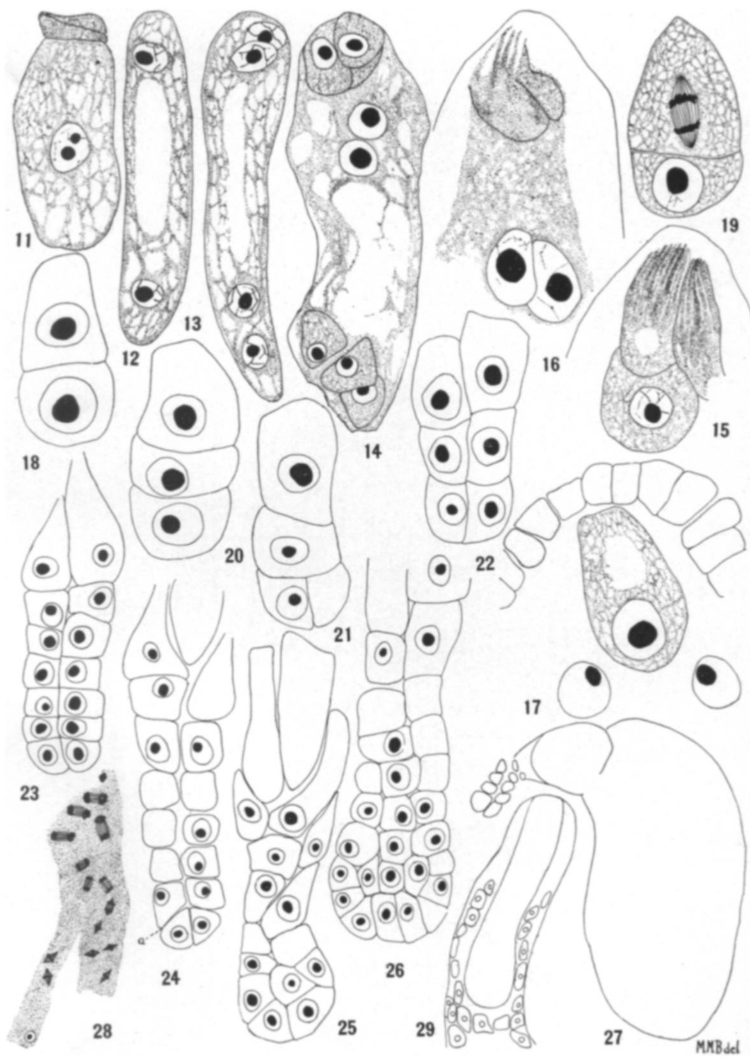


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